Supporting Information

To

Single-Molecule Kinetics Reveals a Hidden Surface Reaction Intermediate in Single-Nanoparticle Catalysis

Hao Shen,† Xiaochun Zhou, ‡ Ningmu Zou and Peng Chen*

Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, USA.

† Present address: Suzhou Institute of Nano-Tech and Nano-Bionics, Chinese Academy of Sciences, 398 Ruoshui Rd, Suzhou Industrial Park, Suzhou, Jiangsu 215123, P.R. China.

‡ These authors contributed equally.

* Corresponding Author: pc252@cornell.edu

Table of Content

1. Au nanocatalysts preparation and characterization ................................................................. 2
   1.1. Preparation of Au@mSiO₂ nanorods .............................................................................. 2
   1.2. Structural characterization of Au nanocatalysts ............................................................. 2
   1.3. Determining the lengths of Au nanorod core from the SEM images of individual Au@mSiO₂ nanorods ................................................................. 3

2. Analysis procedures to extract the fluorescence intensity vs time trajectories during catalysis of single nanocatalysts .................................................................................. 3
   2.1. Analysis procedures for 5.3 nm pseudospherical Au nanoparticles ............................... 4
   2.2. Analysis procedures for Au@mSiO₂ nanorods ............................................................... 4
   2.3. Distribution of fluorescence burst on-time during catalysis by Au nanocatalysts ......... 4

3. The distributions of τ, once compiled from many nanocatalysts, lose the characteristic rise-followed-by-decay behavior from the kinetic intermediate .......................................... 5
   3.1. Distributions of τ compiled from many Au@mSiO₂ nanorods ......................................... 5
   3.2. Distributions of τ compiled from many 5.3 nm Au nanoparticles .................................. 5

4. Kinetic mechanism and derivation of the probability density function f(τ) of the microscopic reaction time τ ..................................................................................................................... 6
   4.1. Mechanism 1: Surface-adsorbed AR● being the kinetic intermediate ............................ 8
   4.2. Mechanism 2: Surface-adsorbed OH● being the kinetic intermediate .......................... 11

5. References ............................................................................................................................. 14
1. Au nanocatalysts preparation and characterization

1.1. Preparation of Au@mSiO₂ nanorods

Au@mSiO₂ nanorods were made by first synthesizing the Au nanorods via seeded growth (following Murphy et al.¹), then coating the nanorods with a silica shell, and then base etching the shell to make it mesoporous, as reported in detail by us previously.²

For the seeded growth to make Au nanorods, pseudospherical Au seeds were first prepared by adding 0.6 mL 0.1 M of ice cold NaBH₄ into 20 mL aqueous solution containing 2.5 × 10⁻⁴ M HAuCl₄ (Aldrich) and 2.5 × 10⁻⁴ M trisodium citrate (J. T. Baker). To grow the nanorods, 1 mL of the as-prepared seed solution was added into three identical growth solutions constituted by 2.5 × 10⁻⁴ M HAuCl₄, 0.1 M cetyltrimethylammonium (CTAB) (Aldrich), and 5.6 × 10⁻⁴ M ascorbic acid (EM Science) sequentially. The volumes of the growth solutions were 9, 18 and 180 mL, respectively, while the growth times were 15 s, 30 s, and overnight.

To grow the silica shell on the nanorods, a freshly prepared 150 µL ethanol solution of 0.54 mM 3-mercaptopropyltrimethoxysilane (MPTMS) was added to 4 mL of the final Au nanorod solution with vigorous stirring. After 30 min, 150 µL of 0.54% w/v aqueous Na₂SiO₃ solution was added and stirred for another 48 hours to form a thin layer of silica on Au nanorods. Further growth of the silica shell was done by adding 160 µL, 10 v% tetraethyl orthosilicate (TEOS) (Aldrich) under stirring, following the procedure by Stöber et al.³ The silica-coated Au nanorods were then etched with NaOH in the presence of CTAB to make the shell mesoporous. 70 µL 0.1 M NaOH and 140 µL 0.1 M CTAB was added under stirring at room temperature for 15 min. The solution was then transferred to 363 K oil bath and left unstirred for 2 hours. After recovering the Au@mSiO₂ nanorods by centrifugation, they were first heated in air at 373 K for 2 hours, then calcinated at 773 K for another 1 hour to remove the capping ligands and other organic components while the Au nanorod cores maintain their morphology as analyzed by TEM.²

1.2. Structural characterization of Au nanocatalysts

The Au nanocatalysts were structurally characterized by TEM. Figure S1A (same as Figure 1C in the main text) is the TEM of pseudospherical Au nanoparticles (Ted Pella JME1052). The averaged diameter is measured to be 5.3 ± 0.7 nm (Figure S1B). For the Au@mSiO₂ nanorods, the lengths of Au nanorod cores are poly-disperse, ranging from ~100 nm to 600-700 nm (Figure S1C, and Figure 1B in the main text), but their diameters are monodisperse, as we reported previously:² the averaged diameter of the Au core of samples used in this study is 21.4 ± 3.2 nm, with the mSiO₂ shell about 80 nm in thickness.² The Au@mSiO₂ sample usually is a mixture of nanorods, nanoparticles and nanoplates (Figure S1C), but the nanorods are easily identified in our single-molecule imaging measurements as well as in the parallel SEM images (e.g., Figure S2 below).⁴
Figure S1. (A) TEM image of pseudospherical 5.3 nm Au nanoparticles. (B) Distribution of nanoparticle diameter from A. Red curve is a Gaussian fit. (C) TEM image of mSiO₂ coated Au nanorods coexisting with nanoplates and nanoparticles.

1.3. Determining the lengths of Au nanorod core from the SEM images of individual Au@mSiO₂ nanorods

The nanorod length used in the main text (Figure 5A-C and section 3.5) refers to the length of the Au nanorod core of a Au@mSiO₂ nanorod. It was determined from the SEM image (Figure S2) of the same sample after the single-molecule catalysis imaging measurements, from which the overall length (including the mSiO₂ shell) was directly determined. With the known mSiO₂ shell thickness (~80 nm) from TEM characterization, the length of the Au nanorod core was then deduced. The details about the combined TEM and SEM characterization of the Au@mSiO₂ nanorods were reported in our previous publication.²

Figure S2. (A) SEM image of Au@mSiO₂ nanorods after single-molecule catalysis imaging measurements. The individual nanorods are clearly identifiable. (B) The SEM image for the particular Au@mSiO₂ nanorod, whose catalytic data were presented in Figure 2B-C and Figure 3 in the main text. The red line outlines its contour, which is the same contour as in Figure 3A in the main text.

2. Analysis procedures to extract the fluorescence intensity vs time trajectories during catalysis of single nanocatalysts

The 5.3 nm pseudospherical Au nanoparticles are invisible in fluorescence microscopy owing to their small size. Under catalysis, the detected emission signal comes entirely from the laser-induced fluorescence of the catalytic reaction product resorufin. On the other hand, the Au nanorods within Au@mSiO₂ nanorods have intrinsic stable emission under laser excitation,²,⁵-⁷
and the fluorescence of the reaction products during catalysis appears on top of a constant nanorod emission background. Therefore, two different analysis procedures were used to analyze the fluorescence movies to extract the fluorescence intensity versus time trajectories for individual 5.3 nm pseudospherical Au nanoparticle and Au@mSiO₂ nanorods, respectively. The analysis procedures are each described below.

2.1. Analysis procedures for 5.3 nm pseudospherical Au nanoparticles

A simple, low computational cost procedure was used here, as described previously.⁸⁻¹⁰ A home-written IDL program was used to extract fluorescence trajectories throughout the entire movie. Intensities of localized fluorescence spots were obtained by integrating the signal counts over a ~7 × 7 pixel area (each pixel ~267 nm). Integration over different fluorescence spots were running parallel to each other, giving an output file containing all the fluorescence trajectories from the many individual nanoparticles in the movie. For each single trajectory, the microscopic reaction time $\tau$ was extracted as the time between a fluorescent burst and its subsequent one (reference Figures 2A and 3A in the main text).

2.2. Analysis procedures for Au@mSiO₂ nanorods

The analysis of the fluorescence signals from individual Au@mSiO₂ nanorods was described in detail in our previous publication.² Briefly, the fluorescence trajectory for each Au@mSiO₂ nanorod during catalysis was first obtained by integrating fluorescence counts within a ~7 × 7 pixel area, which is sufficiently large to encompass the entire nanorod as well as the fluorescence signal of the catalytic product. A two-stage fluorescence intensity thresholding procedure was used, as described in detail previously,² to select fluorescence signals from the catalytic products while suppressing nanorod emission contributions. The image frames corresponding to the selected fluorescence bursts were further analyzed. The intrinsic emission signal from the nanorod was first subtracted and the remaining fluorescence image (in a 13×13 pixel area) was fitted with a 2D Gaussian function (as an approximation to the fluorescence point spread function, PSF) on top of a sloping background. This fitting generates the center position ($x₀, y₀$) of the fluorescent molecule, the errors/uncertainties ($Eₓ, Eᵧ$) of the center position, and the widths ($σₓ, σᵧ$) of the PSF. Based on the PSF fitting results, the fluorescence bursts selected from the prior intensity thresholding were further filtered by two criteria: (1) The errors of the center position in PSF fitting, $Eₓ, Eᵧ$, should be less than 1000 nm; this removes the signals having poor signal-to-noise ratios. (The average $Eₓ$ and $Eᵧ$ is about 10-20 nm.) (2) $σₓ$ and $σᵧ$, the widths of the fitted PSF, should be $80 \text{ nm} < σₓ, y < 1000 \text{ nm}$, as the rest are unreasonable for the fluorescence PSF of a single molecule. (The average $σₓ$ and $σᵧ$ is about 180 nm.) After these additional filtering, the final catalytic fluorescence events were registered, including the timing of each event and the location of each catalytic product molecule. The time separations, i.e., $τ$, between temporally neighboring events were determined.

2.3. Distribution of fluorescence burst on-time during catalysis by Au nanocatalysts

The duration (i.e., on-time) of each fluorescent bursts represents the waiting time for a product resorufin molecule to fully dissociate from the nanocatalyst. The average on-time for Au@mSiO₂ nanorods and 5.3 nm Au nanoparticles are around 100 ms and 50 ms, respectively (Figure S3A and B), which are much shorter than their respective average $τ$. In this study, on-time was not analyzed separately and was included in $τ$. 

S4
Figure S3. Distribution of fluorescence burst on-time from: (A) Compiled ~ninety Au@mSiO₂ nanorods at [AR] = 50 nM; (B) Compiled ~ninety 5.3 nm Au nanoparticles at [AR] = 1.2 µM. [H₂O₂] was kept at 60 mM for both (A) and (B).

3. The distributions of τ, once compiled from many nanocatalysts, lose the characteristic rise-followed-by-decay behavior from the kinetic intermediate

3.1. Distributions of τ compiled from many Au@mSiO₂ nanorods

Figure S4 shows the distributions of τ compiled from 90 Au@mSiO₂ nanorods at two different [AR]. Compared with the distribution of τ from a single Au@mSiO₂ nanorod (e.g., Figure 2B, C and D in the main text), the initial rise part of the τ distribution is indiscernible, and the distributions show apparent single-exponential decay behavior, which is most often seen in single-molecule kinetics. This indiscernibility results from the heterogeneity in catalytic kinetics among the nanorods, and it indicates that even with single-turnover resolution, single-particle level measurements are needed as well to unmask the kinetic intermediate.

Figure S4. Distribution of τ compiled from 90 Au@mSiO₂ nanorods at [AR] = 50 nM (A) and [AR] = 100 nM (B). H₂O₂ concentration was kept at 60 mM. Solid red lines are fits with $y = A \cdot e^{-kr}$.

3.2. Distributions of τ compiled from many 5.3 nm Au nanoparticles

Figure S5 shows the distributions of τ compiled from two sets of 5.3 nm Au nanoparticles. Each set contains ~80 nanoparticles. Again the initial-rise-and-then-decay behavior observed from a single 5.3 nm Au nanoparticle (Figure 3B in the main text) cannot be clearly discerned in
the compiled data. The distributions only contain the decay part, which can be well fitted with a single-exponential decay function.

![Graph of τ distribution](image)

**Figure S5.** Distribution of \( \tau \) compiled from ~eighty 5.3 nm Au nanoparticles at \([AR] = 0.5 \mu M\) (A) and \([AR] = 1.2 \mu M\) (B). H\(_2\)O\(_2\) concentration was kept at 60 mM. Solid red lines are fits with \( y = A \cdot e^{-k\tau} \).

4. Kinetic mechanism and derivation of the probability density function \( f(\tau) \) of the microscopic reaction time \( \tau \)

Our previous study showed that amplex red oxidation by H\(_2\)O\(_2\) generates the resorufin and acetate, in amplex-red:H\(_2\)O\(_2\):resorufin:acetate = 1:1:1:1 stoichiometry, which is described by the balanced chemical equation in Scheme S1. Overall, it involves a two-electron oxidation of amplex red.

**Scheme S1.** Balanced chemical equation of oxidative deacetylation of amplex red by H\(_2\)O\(_2\) to generate resorufin, acetate, and water
Scheme S2. Possible mechanism of catalysis

\[
\begin{align*}
AR + H_2O_2 & \quad \xrightarrow{i} \quad MA_nB_{2n} \quad \xrightarrow{ii} \quad MA_nH_n, \\
& \text{Possible mechanism of catalysis} \quad \text{Possible mechanism of catalysis} \\
& \quad \xrightarrow{iii} \quad MA_{m-1}B_{2n-1}^{-1} \quad \xrightarrow{iv} \quad MA_{m-1}B_{2n-2}^{-*P} \quad \xrightarrow{v} \quad MA_{m-1}B_{2n-2}^{-P}.
\end{align*}
\]

\(^a\) M: Au nanocatalyst; A: amplex red; H: H\(_2\)O\(_2\); OH\(^•\): radical; I: the intermediate AR\(^•\); P*: resorufin molecule in a possibly fluorescence-quenched state; P: fluorescent product resorufin. MA\(_n\)B\(_{2n}\) represents a Au nanorod/nanoparticle having \(m\) adsorbed amplex red molecules and \(2n\) OH\(^•\).

Scheme S2 presents a possible reaction sequence for surface-catalyzed oxidative deacetylation of amplex red to resorufin on Au nanocatalysts, which has 1:1 reaction stoichiometry between amplex red and \(H_2O_2\). The two reactants first adsorb reversibly to the Au surface (step \(i\)). The adsorbed \(H_2O_2\) then undergoes a reversible hemolytic O-O bond cleavage to generate surface-bound OH\(^•\) radicals (step \(ii\)). The presence of OH\(^•\) radicals in the reaction pathway is supported by our observation that the catalytic rate is strongly quenched by DMSO (Figure 4B in the main text), a known effective OH\(^•\) quencher.\(^{11-16}\) It is also known that Au surface can catalyze \(H_2O_2\) decomposition to form OH\(^•\) radicals,\(^{17-23}\) and this reaction is likely reversible.\(^{23}\) Then the reaction proceeds via a one-electron oxidation of AR by a surface bound OH\(^•\) radical, generating a radical species AR\(^•\) (step \(iii\)). A subsequent 2\(^{nd}\) electron oxidation generates the product resorufin (step \(iv\)). The deacetylation could be accompanying the 1\(^{st}\) or the 2\(^{nd}\) oxidation step, or occur as a separate step that is not explicitly included here. Although our previous studies on small (\(\leq 15\) nm in diameter) bare Au nanoparticles showed that we could directly detect the fluorescence of P on their surfaces, for the Au@mSiO\(_2\) nanorods here, the generated P on the Au nanorod surface could potentially be fluorescently quenched (i.e., P*) due to the proximity to the Au surface, before desorbing from Au nanorod surface (step \(v\)) and getting temporarily trapped in the mSiO\(_2\) shell of the Au@mSiO\(_2\) nanorods. We will justify below that P*, the fluorescence-quenched species, either does not exist in the reaction pathway or at least does not exist in a kinetically relevant fashion (i.e., step \(v\) is very fast).

The distributions of the microscopic reaction time \(\tau\) from our single-molecule catalysis measurements (Figures 2B-D and Figures 3B-C in the main text) have shown that the reaction kinetics contains two rate-determining steps straddling over one kinetic intermediate. In Scheme S2, step \(i\) can be assumed to follow a fast adsorption-desorption equilibrium, in which amplex red and \(H_2O_2\) each follow a Langmuir adsorption behavior onto different types of surface sites, which would be consistent with the saturation kinetics observed with increasing reactant concentrations (Figure 2G, Figure 3F, Figure 4A and Equation (S16) later). The fluorescence-quenched P* species on the Au surface could not be the observed kinetic intermediate; otherwise, the desorption of P* to become P that is temporarily trapped in the mSiO\(_2\) shell would be one of the rate-determining steps, but this step does not exist for the bare 5.3 nm Au nanoparticles for which...
the kinetic intermediate is still observed (Figure 3B-C in the main text). Thus, step v in Scheme S2 is not a kinetically relevant step, and is not included in the mechanism in Scheme 1 in the main text.

Therefore, the two rate-determining steps observed experimentally should come from the remaining three reaction steps in Scheme S2 (i.e., step ii, iii, and iv), involving two possible reaction intermediates, the one-electron-oxidized amplex radical AR• and the OH• radical. The involvement of the AR• radical in the oxidation of amplex red to form resorufin has been shown in the catalysis by the enzyme horseradish peroxidase.24-27 The involvement of OH• radical in the reaction is supported by our observation that DMSO can quench the reaction (Figure 4B in the main text). Below we will treat the two possibilities separately. And later we will present evidences that argue against OH• being the reaction kinetic intermediate observed in our single-molecule kinetics.

4.1. Mechanism 1: Surface-adsorbed AR• being the kinetic intermediate. Assuming the amplex red radical (AR•) being the observed reaction intermediate, steps iii and iv are then the two rate-determining steps, with all other steps being fast in Scheme S2. Consequently, the reaction mechanism reduces to the effective kinetic mechanism in Scheme S3 with only two kinetic steps:

### Scheme S3. Reduced effective kinetic mechanism with AR• being the observed reaction intermediate

\[
MA_mB_{2n} \xrightarrow{k_{app1}} MA_{m-1}B_{2n-1}^{-1} \xrightarrow{k_{app2}} MA_{m-2}B_{2n-2}^{-1}P
\]

Here MA_mB_{2n} is a Au nanocatalyst (i.e., M) with surface adsorbed amplex red (i.e., A) and HO• radical (i.e., B) in rapid equilibrium with amplex red and H_2O_2 in solution. I stands for the amplex red radical AR•, P for the final product resorufin. m is the number of amplex red molecule adsorbed on the catalyst particle surface and 2n is the number of OH• radical adsorbed. k_{app1} and k_{app2} are the two corresponding apparent rate constants. The first step involves amplex red; its associated apparent rate constant k_{app1} is expected to be dependent on the concentration of amplex red on the catalytic surface and thus on its concentration in solution. The second step is expected to be independent of amplex red concentration. Our experimental results in Figure 2E-F and Figure 3D-E in the main text show that the amplex-red-concentration-independent step (which corresponds to k_{app2} here; see the derived Equation (S8) below) is one magnitude faster than the amplex-red-concentration-dependent step (which corresponds to k_{app1} here; see Equation (S7) below). Therefore, in Scheme S3, the formation of the intermediate I is slower, and it is followed by a faster consumption step. Therefore, we assume no intermediate accumulation on catalytic surface.

The apparent rate constants k_{app1} and k_{app2} take the following forms:

\[k_{app1} = \gamma_1 m(2n) \quad \text{(S1)}\]

\[k_{app2} = \gamma_2(2n - 1)(1) \approx \gamma_2(2n) \quad \text{(S2)}\]
where $\gamma_1$ and $\gamma_2$ are rate constants.

Assuming noncompetitive adsorption between amplex red and $\text{H}_2\text{O}_2/\text{HO}^\bullet$ on the nanoparticle surface (i.e., they adsorb onto different types of surface sites), Langmuir adsorption model gives:

$$m = n_A \theta_A$$  
(S3)

$$2n = n_B \theta_B$$  
(S4)

where $n_A$ and $n_B$ are the total number of adsorption sites for amplex red and HO• respectively; $\theta_A$ and $\theta_B$ are the respective fractions of surface sites occupied by amplex red and OH•. For simple unimolecular Langmuir adsorption of amplex red, $\theta_A$ takes the form:

$$\theta_A = \frac{G_A[A]}{1 + G_A[A]}$$  
(S5)

where $G_A$ is the adsorption equilibrium constant of amplex red and $[A]$ is the amplex red concentration in solution. On the other hand, HO• radical comes from the O-O bond cleavage of $\text{H}_2\text{O}_2$ upon adsorption, and each $\text{H}_2\text{O}_2$ molecules generates two OH•; here $\theta_B$ takes the form:

$$\theta_B = \frac{G_B^{1/2}[H]^{1/2}}{1 + G_B^{1/2}[H]^{1/2}}$$  
(S6)

In Equation (S6), $G_B$ represents the equilibrium constant for $\text{H}_2\text{O}_2$ adsorbing to directly become two adsorbed HO• (i.e., steps $i$ and $ii$ in Scheme S2 collapse into one effective adsorption equilibrium, with minimal $\text{H}_2\text{O}_2$ adsorbed as intact molecules on Au surfaces), and $[H]$ is the $\text{H}_2\text{O}_2$ concentration in solution. Therefore,

$$k_{\text{app}1} = \gamma_1 n_A n_B \frac{G_A[A]}{1 + G_A[A]} \frac{G_B^{1/2}[H]^{1/2}}{1 + G_B^{1/2}[H]^{1/2}} = \gamma_{\text{eff}1} \frac{G_A[A]}{1 + G_A[A]} \frac{G_B^{1/2}[H]^{1/2}}{1 + G_B^{1/2}[H]^{1/2}}$$  
(S7)

$$k_{\text{app}2} = \gamma_2 n_B \frac{G_B^{1/2}[H]^{1/2}}{1 + G_B^{1/2}[H]^{1/2}} = \gamma_{\text{eff}2} \frac{G_B^{1/2}[H]^{1/2}}{1 + G_B^{1/2}[H]^{1/2}}$$  
(S8)

where $\gamma_{\text{eff}1} = \gamma_1 n_A n_B$ and $\gamma_{\text{eff}2} = \gamma_2 n_B$. At saturating $\text{H}_2\text{O}_2$ concentrations, $\frac{G_B^{1/2}[H]^{1/2}}{1 + G_B^{1/2}[H]^{1/2}} \rightarrow 1$, Equations (S7) and (S8) reduces to:

$$k_{\text{app}1} = \gamma_{\text{eff}1} \frac{G_A[A]}{1 + G_A[A]}$$  
(S9)

$$k_{\text{app}2} = \gamma_{\text{eff}2}$$  
(S10)

The single-molecule probabilistic rate equations for Scheme S3 are:

$$\frac{dP_{\text{MA}_m\text{B}_{2n}}}{dt} = -k_{\text{app}1}P_{\text{MA}_m\text{B}_{2n}}$$  
(S11)

$$\frac{dP_{\text{MA}_{m-1}\text{B}_{2n-1}}}{dt} = k_{\text{app}1}P_{\text{MA}_m\text{B}_{2n}} - k_{\text{app}2}P_{\text{MA}_{m-1}\text{B}_{2n-1}}$$  
(S12)
\[
\frac{dP_{MA_m B_{2n-2}}}{dt} = k_{app2} P_{MA_m B_{2n-1} - 1}
\]

(S13)

where \( P \) represents the probability of finding the nanoparticle at each state. The initial conditions are \( P_{MA_m B_{2n}} = 1, P_{MA_m B_{2n-1} - 1} = 0, \) and \( P_{MA_m B_{2n-2}} = 0; \) and the boundary condition is \( P_{MA_m B_{2n}} + P_{MA_m B_{2n-1} - 1} + P_{MA_m B_{2n-2}} = 1. \)

If \( f(\tau) \) is the probability density function of the microscopic reaction time \( \tau, \) \( f(\tau) \Delta \tau \) is the probability of finding a particular \( \tau. \) This probability is equal to the probability of switching from \( P_{MA_m B_{2n-1} - 1} \) to \( P_{MA_m B_{2n-2}} \) between \( t = \tau \) and \( t = \tau + \Delta \tau, \) which is \( \Delta P_{MA_m B_{2n-2}}. \)

Then \( \langle \tau \rangle \) is the time-averaged product formation rate, which is the equivalent to the rate of turnovers, for a single catalyst particle is:

\[
\langle \tau \rangle^{-1} = 1 / \int_{0}^{\infty} \tau f(\tau) \, d\tau = \frac{Y_{eff1}G[A]}{Y_{eff2} + (Y_{eff2} - Y_{eff1})G[A]} \cdot \frac{Y_{eff2}G_B^{1/2}[H]^{1/2}}{1 + G_B^{1/2}[H]^{1/2}} \cdot \left( \exp \left( -Y_{eff1} \frac{G[A]}{1 + G_A[A]} \frac{G_B^{1/2}[H]^{1/2}}{1 + G_B^{1/2}[H]^{1/2}} \right) \right) - \exp \left( -Y_{eff2} \frac{G_B^{1/2}[H]^{1/2}}{1 + G_B^{1/2}[H]^{1/2}} \right)
\]

(S16)

Note that Equation (S14) has the same form as the empirical equation \( y = A \cdot \left( e^{-k_1 \tau} - e^{-k_2 \tau} \right) \) (Equation (1) in the main text) used in fitting Figure 2B-D and Figure 3B-C in the main text. Therefore, \( k_{app1} \) and \( k_{app2} \) correspond to \( k_1 \) and \( k_2 \) for this mechanism.

Replacing \( k_{app1} \) and \( k_{app2} \) using equations (S7)-(S8):

\[
f(\tau) = \frac{Y_{eff1}G[A]}{Y_{eff2} + (Y_{eff2} - Y_{eff1})G[A]} \cdot \frac{Y_{eff2}G_B^{1/2}[H]^{1/2}}{1 + G_B^{1/2}[H]^{1/2}} \cdot \left( \exp \left( -Y_{eff1} \frac{G[A]}{1 + G_A[A]} \frac{G_B^{1/2}[H]^{1/2}}{1 + G_B^{1/2}[H]^{1/2}} \right) \right) - \exp \left( -Y_{eff2} \frac{G_B^{1/2}[H]^{1/2}}{1 + G_B^{1/2}[H]^{1/2}} \right)
\]

(S15)

Then \( \langle \tau \rangle^{-1}, \) the time-averaged product formation rate, which is the equivalent to the rate of turnovers, for a single catalyst particle is:

\[
\langle \tau \rangle^{-1} = 1 / \int_{0}^{\infty} \tau f(\tau) \, d\tau = \frac{Y_{eff1}G_A[A]}{Y_{eff2} + (Y_{eff2} - Y_{eff1})G_A[A]} \cdot \frac{Y_{eff2}G_B^{1/2}[H]^{1/2}}{1 + G_B^{1/2}[H]^{1/2}}
\]

(S16)

When the H\(_2\)O\(_2\) concentration is saturating (i.e., \( [H] \to \infty \)), which is our experimental condition for Figures 2 and 3 in the main text, \( \frac{G_B^{1/2}[H]^{1/2}}{1 + G_B^{1/2}[H]^{1/2}} \to 1. \) Equations (S15) simplifies to:

\[
f(\tau) = \frac{Y_{eff1}G_A[A]}{1 + (1 - \frac{Y_{eff2}}{Y_{eff1}})G_A[A]} \cdot \left( \exp \left( -Y_{eff1} \frac{G_A[A]}{1 + G_A[A]} \tau \right) \right) - \exp \left( -Y_{eff2} \tau \right)
\]

(S17)

\( f(\tau) \) is now a bi-exponential function, with one exponent dependent on \( [A] \) (i.e., the amplex red concentration) and the other one independent of \( [A], \) as we observed experimentally (Figure 2E-F and Figure 3D-E in the main text).
For $\langle \tau \rangle^{-1}$, at $[H] \to \infty$, it becomes

$$\langle \tau \rangle^{-1} = \frac{\gamma_{eff1} G_A[A]}{1 + \left( 1 + \frac{\gamma_{eff1}}{\gamma_{eff2}} \right) G_A[A]} \frac{\gamma_{eff2} \gamma_{eff1}}{1 + G_A[A]}$$ \hfill (S18)

$\langle \tau \rangle^{-1}$ represents simple saturation kinetics, and it equals $\gamma_{eff1}/(1+\gamma_{eff}/\gamma_{eff2})$ when $[A] \to \infty$. And when $[A] \to \infty$ and $\gamma_{eff2} \gg \gamma_{eff1}$, $\langle \tau \rangle^{-1} = \gamma_{eff1}$.

When $[H] \to \infty$ and $[A] \to \infty$, $G_A[A]/\left( 1 + G_A[A] \right) \to 1$ and $G_B^{1/2}[H]^{1/2}/\left( 1 + G_B^{1/2}[H]^{1/2} \right) \to 1$. We have then:

$$f(\tau) = \frac{\gamma_{eff1} \gamma_{eff2}}{\gamma_{eff2} - \gamma_{eff1}} \cdot \left\{ \exp(-\gamma_{eff1} \tau) - \exp(-\gamma_{eff2} \tau) \right\}$$ \hfill (S19)

$$\langle \tau \rangle^{-1} = \frac{\gamma_{eff1} \gamma_{eff2}}{\gamma_{eff2} + \gamma_{eff1}} \to \gamma_{eff1}$$ \hfill (S20)

4.2. Mechanism 2: Surface-adsorbed OH\textsuperscript{•} being the kinetic intermediate. In the last section, we presented the mechanism assuming the amplex red radical being the observed reaction intermediate. Here we present the alternative mechanism assuming OH\textsuperscript{•} radical being the observed reaction intermediate. Then, in Scheme S2, the two rate-determining steps would be steps \textit{ii} and \textit{iii}, i.e., the O-O bond cleavage of H\textsubscript{2}O\textsubscript{2} to form two OH\textsuperscript{•} radicals that would be independent of amplex red, and the first 1-electron oxidation of amplex red by OH\textsuperscript{•} that would be dependent on the amplex red concentration on the catalyst surface, before eventually generating the product resorufin. Consequently, the kinetic scheme simplifies to having only two kinetic steps (Scheme S4), where MA\textsubscript{m}H\textsubscript{n} is a Au nanocatalyst (i.e., M) with surface adsorbed amplex red (i.e., A) and H\textsubscript{2}O\textsubscript{2} (i.e., H) in rapid equilibrium with amplex red and H\textsubscript{2}O\textsubscript{2} in solution.

\textbf{Scheme S4}. Reduced kinetic mechanism with OH\textsuperscript{•} being the observed reaction intermediate

In this mechanism, as the O-O bond cleavage is assumed to be one of the rate-determining steps (i.e., relatively slow), only part of the adsorbed H\textsubscript{2}O\textsubscript{2} (i.e., H) will convert into OH\textsuperscript{•} radical (i.e., B). In another word, the adsorption sites for H\textsubscript{2}O\textsubscript{2} will now either be occupied by H\textsubscript{2}O\textsubscript{2}, or OH\textsuperscript{•}, or be vacant. The formation of OH\textsuperscript{•} would require a surface adsorbed H\textsubscript{2}O\textsubscript{2} and a nearby vacant site. Therefore, the apparent rate constant $k_{app1}$ would take the following form:\textsuperscript{28}

$$k_{app1} = \gamma_1 \cdot n_H \theta_H \cdot n_B(1 - \theta_B - \theta_H)$$ \hfill (S21)
Here \( n_H \) is the total number of adsorption sites for H\(_2\)O\(_2\) and OH•; \( \theta_H \) and \( \theta_B \) are the fractions of sites occupied by H\(_2\)O\(_2\) and OH• radical respectively, and \( \gamma \) is a rate constant.

The apparent rate constants \( k'_{\text{app1}} \) and \( k_{\text{app2}} \) take the following forms:

\[
k'_{\text{app1}} = \gamma'(n_H\theta_B)^2
\]

\[
k_{\text{app2}} = \gamma_2n_H\theta_Bn_A\theta_A
\]

Here \( \gamma' \) and \( \gamma_2 \) are two rate constants. \( n_A \) and \( \theta_A \) are the number of adsorption sites for amplex red and their fractional occupation, as defined previously, and \( \theta_A = \frac{G_A[A]}{1+G_A[A]} \) as in Equation (S5). Note for \( k_{\text{app2}} \), we assume this rate-determining step is first order to the surface concentration of the OH• radical because the overall 2-electron oxidation of amplex red likely goes through two sequential 1-electron processes with the 1st one being the rate-determining as described in Scheme S2 and Mechanism 1 earlier.

Applying steady-state approximation for the surface concentration of the intermediate OH•, [OH•], we have:

\[
\frac{d[\text{OH•}]}{dt} = 2\gamma_1n_H^2\theta_H(1 - \theta_H - \theta_B) - \gamma'(n_H\theta_B)^2 - \gamma_2n_H\theta_Bn_A\theta_A = 0
\]

As H\(_2\)O\(_2\) is assumed to be in rapid adsorption/desorption equilibrium between in solution and on the catalyst surface, we have

\[
k_{\text{ads}}[H]n_H(1 - \theta_H - \theta_B) = k_{\text{des}}n_H\theta_H
\]

Here \( k_{\text{ads}} \) and \( k_{\text{des}} \) are the adsorption and desorption rate constants for H\(_2\)O\(_2\), respectively, and [H] is the concentration of H\(_2\)O\(_2\) in solution. Combine (S24) and (S25), and let \( G_H = k_{\text{ads}}/k_{\text{des}} \), we have:

\[
y'n_H^2\theta_B^2 + \gamma_2n_An_H\theta_A\theta_B = 2\gamma_1n_H^2 \frac{G_H[H]}{(1 + G_H[H])^2}(1 - \theta_B)^2
\]

Let \( a = 2\gamma_1n_H^2 \frac{G_H[H]}{(1 + G_H[H])^2} \), \( b = \gamma'n_H^2 \), and \( c = \gamma_2n_An_H\theta_A = \gamma_2n_An_H \frac{G_A[A]}{1+G_A[A]} \), Equation (S26) becomes:

\[
(a - b)\theta_B^2 - (2a + c)\theta_B + a = 0
\]

Considering \( \theta_B \) represents the fraction of active sites covered by OH• radical, then \( 0 < \theta_B < 1 \). Solving Equation (S27) gives:

\[
\theta_B = \frac{2a + c - \sqrt{4ac + 4ab + c^2}}{2a - 2b}
\]

Substitute into Equation (S25), we have:

\[
\theta_H = \frac{G_H[H]}{1 + G_H[H]} \cdot \frac{2b + c - \sqrt{4ac + 4ab + c^2}}{2b - 2a}
\]

Therefore, for \( k_{\text{app1}}, k'_{\text{app1}}, \) and \( k_{\text{app2}} \) in Scheme S4, they are:
The probability density function \( f(\tau) \) for the microscopic reaction time \( \tau \) corresponding to Scheme S4 has been derived in our previous publication\textsuperscript{29} and earlier by others as well:\textsuperscript{30-32}

\[
f(\tau) = \frac{k_{\text{app1}}k'_{\text{app1}}}{2\alpha} [e^{\beta+\alpha}\tau - e^{\beta-\alpha}\tau]
\]

where \( \alpha = \frac{1}{4} (k_{\text{app1}} + k'_{\text{app1}} + k_{\text{app2}})^2 - k_{\text{app1}}k_{\text{app2}} \) and \( \beta = -(k_{\text{app1}} + k'_{\text{app1}} + k_{\text{app2}})/2 \).

\( f(\tau) \) here is again a bi-exponential function, and the two exponents are \( \beta+\alpha \) and \( \beta-\alpha \). If mechanism 2 is the correct mechanism for interpreting our data, \( \beta+\alpha \) and \( \beta-\alpha \) would corresponding to \( k_1 \) and \( k_2 \) in Equation (1) in the main text that was used to empirically fit Figure 2B-D and Figure 3B-C in the main text, respectively.

Based on the expression of \( f(\tau) \) in Equation (S33), this alternative mechanism of assuming \( \text{OH}^\bullet \) being the observed reaction intermediate is disfavored for two reasons. First, since \( c = \gamma_2n_A n_H \frac{g_A[A]}{1+g_A[A]} \), \( k_{\text{app1}}, k'_{\text{app1}} \) and \( k_{\text{app2}} \) are all dependent on the amplex red concentration in solution. These dependences stem from the dependence of the three apparent rate constants on \( \theta_3 \) (Equation (S30)-(S32)), the site occupation fraction of \( \text{OH}^\bullet \) radical; and this \( \theta_3 \) is in turn dependent on the amplex red concentration \( [A] \) (Equation (S28), as the surface adsorbed amplex red reacts with surface \( \text{OH}^\bullet \) to eventually generate the product resorufin. Therefore, the two exponents, \( \beta+\alpha \) and \( \beta-\alpha \), of \( f(\tau) \) are both dependent on the amplex red concentration in solution.

This prediction is in conflict with our experimental observation, because the faster-decay exponent in the distribution of \( \tau \) is independent of amplex red concentration (Figure 2F and 3E in the main text).

Second, in this alternative mechanism, the O-O bond cleavage of \( \text{H}_2\text{O}_2 \) to generate \( \text{OH}^\bullet \) on Au surface is assumed to be slow enough to be rate-determining. However, previous work by Lunsford \textit{et al.}\textsuperscript{23} has shown that the \( \text{H}_2\text{O}_2 \) to hydroxyl radical conversion is fast on Au surfaces. They measured the rate of \( \text{H}_2\text{O}_2 \) decomposition on Au, in which the hydroxyl radical formation was part of the decomposition pathways. The overall rate was measured to be 0.1 \( \mu \text{mol cm}^{-2} \text{s}^{-1} \). This rate would correspond to \( \sim 10^7 \) molecules per nanorod per second for \( \text{Au}@\text{mSiO}_2 \) catalyzed reaction (assuming on average a diameter of 21 nm and a length of 350 nm for the nanorod core) or \( 10^5 \) molecules per particle per second for the pseudospherical 5.3 nm Au particle. These predicted rates would be orders of magnitude faster than the highest reaction rates (~2 \( \text{s}^{-1} \) particle\(^{-1} \) for \( \text{Au}@\text{mSiO}_2 \) nanorods and ~0.04 \( \text{s}^{-1} \) particle\(^{-1} \) for 5.3 nm Au nanoparticles, Figure 2G and 3F in the main text) observed experimentally. Based on these two reasons, this alternative
mechanism assuming surface-adsorbed OH• being the observed reaction intermediate is disfavored.

5. References


